DECLARATION OF JACK GAULDIE, Ph.D.
Examining Group 1635
Patent Application
Docket No. GDI-1CPA1
Serial No. 09/360,199

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	Schnizer, Richard
Art Unit	:	1635
Applicants	:	Gauldie et al.
Serial No.	:	09/360,199
Docket No.	:	GDI-1CPA1
Filed	:	07/23/1999
For	:	Intestinal Gene Therapy

Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION OF JACK GAULDIE, Ph.D.

I Jack Gauldie, Ph.D. hereby declare and say as follows:

THAT, I am employed as Professor and Chairman, Department of Pathology and Molecular Medicine at McMaster University, Hamilton, Ontario, Canada.;

THAT, I earned my Ph.D. in Biological Chemistry in 1968 from University College, University of London UK, a copy of my curriculum vitae is attached hereto as Exhibit A;

THAT, I am one of the above-named Applicants and inventors of the subject matter described and claimed in the above-identified patent application;

THAT, by virtue of my educational and employment background, my attendance at seminars, my ongoing research, my continuing review of scientific periodicals and journals, and through correspondence with professional colleagues, I am aware of the level of skill of one ordinarily skilled in the art of immunology and vaccinology;

THAT, I have studied the application Serial No. 09/360,199 and all office actions which have been issued during prosecution of this application (including cited references), as well as all responses which have been filed on the Applicants' behalf, and being thus duly qualified declare as follows:

1. The Office action questions whether the vaccination methods taught in the specification and claimed in the subject application achieve the desired therapeutic effect of immunizing an animal against a predetermined pathogen. The office action cites a number of references to allege that gene therapy and genetic immunization are unpredictable arts. In particular, the office action questions whether the cytotoxic T cell assay (CTL assay) is reliable enough to establish whether the claimed methods achieve a therapeutic effect. The office action cites Bachmann et al (1994) for the proposition that comparative studies have shown that CTL responses readily detectable after in vitro restimulation may not be detected by any in vivo assay. Citing Bachmann, the office actions states the following: "One should therefore be very cautious not to 'over-interpret' cytotoxicity found only by ^{51}Cr-release after secondary in vitro restimulation; without in vivo confirmation the result may be biologically irrelevant." The office action further states that the "Applicant . . . has not provided sufficient evidence or reasoning to support the position that a protective immune response will be generated against any antigen by the claimed methods or composition." I respectfully disagree with the Examiner's position. Since the publication by Bachmann, a number of gene based vaccine approaches have been developed, primarily aimed at developing anti-tumor immunity, in which protection from tumor challenge is associated with the presence of CTL determined by in vitro secondary expansion of T cells and Cr^{51} CTL assays. Moreover, the details we now supply demonstrate direct protective effects of this immunization protocol and all are associated with CTL detection . There may be some instances, such as those quoted by Bachmann, in which CTL assays after secondary in vitro expansion do not correlate with protection, however, most data recently published show correlation, including the data supplied herein.

2. Although I believe that the CTL assay example provided in the specification is sufficient to support a claim to therapeutic value in the present case, as further evidence, I provide herewith *in vivo* data from two different studies, which unquestionably demonstrate that the claimed methods do indeed immunize against targeted pathogens. These studies are provided as Exhibits B and C. Exhibit B demonstrates that Adenoviral-based gene delivery in the lower GI tract induces antigen-specific immune responses and protection from Tumour challenge, correlating with the presence of CTL positive reactions in spleen cells from immunized animals. Exhibit C demonstrates that Advenoviral based antigen gene delivery to rectal epithelium induces protective local immunity against HSV-2 infection, challenged either by vaginal or rectal administration of the pathogen.. In view of these two studies, there can be no question that the claimed methods, as claimed in Applicants' most recent response filed September 15, 2002, are directed to a useful, therapeutic vaccination methodology.

3. The claims as pending before Applicants' September 15, 2002 amendment were rejected over Wang and Henning (PCT publication and U.S. patents). The amendments to the claims so distinguish the prior art that it cannot be said that the Wang and Henning references anticipate or render obvious the now pending claims. I have carefully reviewed the Wang and Henning references. The Examiner correctly asserts that "neither Wang nor Henning teach a working example of a therapeutic effect," see page 15, paragraph 2 of the last office action. The Henning references disclose a method of introducing nucleic acid into the intestine using naked DNA or using various viral vectors. Henning discloses a few hypothetical examples of introducing DNA into intestinal cells. I point out that none of the examples discuss the use of an adenoviral vector; they are limited to retrovirus vectors, which are of limited use in vivo. Furthermore, Henning provides no working example, either in vitro or in vivo, of a methodology that may act to immunize an animal. Based on the teachings of Henning, one skilled in the art is still left wondering whether cells can be transfected in the intestine in vivo to express a given a gene. One skilled in the art knows no more about whether a gene can be reproducibly expressed in the intestine, much less whether an animal can be immunized against a specific pathogen by expression of a given gene.

There is simply no connection between the method of exposing intestinal cells to a nucleic acid taught by Henning and successfully expressing a gene, whereby such expression leads to a successful vaccination of an animal against a given pathogen. The subject application is the first demonstration, as far as I am aware, that shows successful introduction of a gene into genitourinary epithelial cells using an adenoviral vector, whereby a protein antigen is generated that induces an immune response.

4. With respect to the Wang et al. reference, it discloses a specific study involving the exposure of vaginal mucosa to a non-viral vector expressing HIV-1 envelope proteins. The study shows that exposure to the non-viral based DNA plasmid produces immunoglobulins that showed activity in the in vitro cell-free infection assay. The assay involved taking vaginal washes from treated and non-treated animals and combining the wash with HIV-1/MN cell-free virus. The cell free virus was then combined with MT-2 cells, and the ability of the virus to infect the cells was observed. In some cases, it does appear that something in the vaginal wash affects the ability of the virus to infect the MT-2 cells. It is conjectured by Wang et al. that it is immunoglobulins present in the vaginal wash that is affecting the ability of the cell-free virus to infect the MT-2 cells. This study provides little additional information over Henning as to whether a given viral vector is able to be introduced into mucosal cells, express a gene of interest, and induce a protective immune response against a given pathogen. There is the suggestion that it may be worthwhile to study different routes of administration using different vectors. However, in view of either Henning or Wang, it cannot be said that any given route of administration, using non viral or viral vectors, would vaccinate a treated animal with a reasonable expectation of success. The Applicants of the present application are the first to demonstrate that specific vaccination is achievable through gastrointestinal or genitourinary routes by application of an adenoviral vector encoding a specific antigen gene..

5. The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information in belief are believed to be true; and further that these statements were made with the knowledge that willful false statements in the like so made are punishable by fine or imprisonment, or both, under

§1001 of title 18 of the U.S.C. and that such willful false statements made jeopardize the validity of the application or of any patent issuing thereon.

Further declarant sayeth naught.

Jack Gauldie

Jack Gauldie, Ph.D.

Nov 8/02

Date

Exhibit A: Dr. Jack Gauldie's *Curriculum Vitae*

Exhibit B: Adenoviral-based gene delivery in the lower GI tract induces antigen-specific immune responses and protection from Tumour challenge

Exhibit C: Induction of protective distal mucosal immunity against HSV-2 infection

Summary: Induction of protective distal mucosal immunity against HSV-2 infection

Abbreviations:

Adv	Adenoviral vector
IR	Intrarectal
IVAG	Intravaginal
gB	Glycoprotein B
pfu	Plaques forming unit
HSV-2	Herpes simplex virus type 2
tw	Tissue weight
LD_{50}	50% lethal dose
vw	Vaginal wash

Materials and Methods

Animals, cell cultures and viruses

Female C57BL/6 mice were 6-8 weeks of age during immunization with Adv. Vero cells were grown in complete α-MEM media. Recombinant AdgB is an Adenovirus vector that encodes gB8, the surface protein gene from HSV. HSV-2 strain 333 was propagated and titered on Vero cells.

IR immunization and virus challenge

Mice were anesthetized with isoflorane and instilled with 50% ethanol into the colo-rectum, and kept under anesthesia for 30 min. One hour later, AdgB was IR delivered by insertion of a pipet tip into the rectum, followed by one-hour incubation period. 21 days after AdgB IR immunization, mice were IR (rectal challenge) or IVAG (vaginal challenge) challenged with HSV-2 strain 333. For IR challenge, the procedure was as the same as that for IR immunization. For IVAG challenge, mice were injected SC with 2.5 mg of progesterone (Depo-Provera) 5 days prior to administration of HSV-2. 20 μl of HSV-2 was given IVAG, followed by one-hour incubation.

Viral replication and pathology in the anal and genital tract

After IVAG inoculation of HSV-2, vaginal washes were obtained daily by pipetting twice 30 μl of PBS into and out of vaginal tract, and stored at -70°C before use. Virus shedding was determined by plaque assay on Vero cell monolayers and expressed by virus retrieved from per vaginal wash (vw) in 60 μl.

Anal or genital pathology was monitored and scored daily after HSV-2 challenge. Genital pathology was scored by a 6-point scale from 0 to 5 (adapted from Overall et al, 1975; Gallichan et al, 1998, 2001; Kuklin et al, 1998): 0, no change; 1, redness of external vagina; 2, swelling of external vagina, severe redness; 3, perineal hair loss or genital ulceration, severe swelling; 4, perineal ulceration; and 5, hind limb paralysis or death. Anal pathology was also scored on a 6-point scale based on the descriptions by Phillips et al (1998, 2000): 0, no change; 1, redness of anus; 2, swelling of anus, severe redness; 3, perineal hair loss or anal ulceration, severe

swelling of anus and perineum; 4, perineal lesion; and 5, hind limb paralysis, anal restrictions or death.

Results

Dose dependent study of IR challenge of HSV-2

To determine the LD_{50}, naive mice were IR inoculated with HSV-2 strain 333, and monitored for anal pathology (daily for the first 2 weeks). As shown in Table 1, 50% of mice died from HSV-2 IR inoculation at a dose of 2×10^4 pfu. Anal pathology developed rapidly and no mice survived when the doses were increased to 2×10^5 pfu and 2×10^6 pfu. When mice received the latter dose, which is 100-fold higher than LD_{50}, they were all paralyzed by the first week. Because this dose was highly lethal, leading to early onset of anal pathology and rapid death, it was used to challenge AdgB IR-immunized mice.

Table 1. Survival rate (%) of naive mice IR inoculated with HSV-2 (8 mice/group).

Dose (pfu)\week	1	2	3	4
2×10^3	100	75	75	75
2×10^4	87.5	50	50	50
2×10^5	87.5	0	0	0
2×10^6	0	0	0	0

Pathology and survival from IntraRectal HSV-2 challenge in AdgB IR-immunized mice

21 days after a single IR immunization with AdgB, mice were monitored for pathology and survival following a lethal IR challenge of 2×10^6 pfu of HSV-2. All unimmunized mice (n=8) rapidly developed pathology, and were unable to survive the challenge by day 7. In AdgB IR-immunized mice (n=12), 41% of mice had overt pathology and 92% survived the HSV challenge. For those mice that developed pathology, the severity of infection was less than non-immunized mice (maximum score points: 3.6±0.9 vs. 5±0.0), and external indications of infection were no longer visible by week 2, indicating the ability of immunized mice to withstand the infection at high lethal doses.

The development of genital pathology after challenge of 2×10^5 pfu of HSV-2, which is 10-fold higher than conventional dose (2×10^4 pfu), was also assessed. All unimmunized mice died within the first week of challenge, whereas 100% of immunized mice survived. Although 60% of immunized mice demonstrated overt genital pathology (3.7±0.6 vs. 5±0.0), they were also able to control the infection, characterized by regression of some mild perineal lesions.

Virus titers in vaginal washes of IntraVaginal HSV-2 challenge in AgB IR immunized mice

Previous studies have shown that virus shedding peaks at day 3 post infection. Virus shedding was compared on monolayer Vero cells by measuring plaques formed by virus obtained from vaginal washes. Three days after HSV challenge at a dose of 2×10^5 pfu, virus was detected in the samples of all unimmunized mice ($8.0\times10^3\pm3.7\times10^3$ pfu/vw, n=5). Although 80% of immunized mice (n=5) were detected positive for virus shedding, compared to those from unimmunized mice, the virus titers from these immunized mice were at least one log lower ($8.7\times10^2\pm7.0\times10^2$ pfu/vw). While all unimmunized mice retained similar levels of virus shedding until death, 40% of immunized mice were no longer positive for virus by day 5, and all were virus free by day 10.